

CLAIMS

We claim:

- 5 1. A method for detecting the presence of a target nucleic acid comprising:
 - a) cleaving an invasive cleavage structure, said invasive cleavage structure comprising an RNA target nucleic acid; and
 - b) detecting the cleavage of said invasive cleavage structure.
- 10 2. The method of Claim 1, wherein cleaving is carried out by a cleavage agent.
3. The method of Claim 1, wherein said target nucleic acid comprises a first
15 region and a second region, said second region downstream of and contiguous to said first region.
4. The method of Claim 3, wherein said invasive cleavage structure comprises said target nucleic acid, a first oligonucleotide, and a second oligonucleotide,
20 wherein at least a portion of said first oligonucleotide is completely complementary to said first region of said first target nucleic acid, and wherein said second oligonucleotide comprises a 3' portion and a 5' portion, wherein said 5' portion is completely complementary to said second region of said target nucleic acid.
- 25 5. The method of Claim 4, wherein at least said portion of said first oligonucleotide is annealed to said first region of said target nucleic acid and wherein at least said 5' portion of said second oligonucleotide is annealed to said second region of said target nucleic.
- 30 6. The method of Claim 1, wherein said cleaving generates a non-target cleavage product.

7. The method of Claim 6, wherein said detecting the cleavage of said invasive cleavage structure comprises detecting said non-target cleavage product.

8. The method of Claim 4, wherein said 3' portion of said second oligonucleotide comprises a 3' terminal nucleotide not complementary to said target nucleic acid.

9. The method of Claim 4, wherein said 3' portion of said second oligonucleotide consists of a single nucleotide not complementary to said target nucleic acid.

10. The method of Claim 1, wherein said detecting the cleavage of said invasive cleavage structure comprises detection of fluorescence.

11. The method of Claim 1, wherein said detecting the cleavage of said invasive cleavage structure comprises detection of mass.

12. The method of Claim 1, wherein said detecting the cleavage of said invasive cleavage structure comprises detection of fluorescence energy transfer.

13. The method of Claim 1, wherein said detecting the cleavage of said cleavage structure comprises detection selected from the group consisting of detection of radioactivity, luminescence, phosphorescence, fluorescence polarization, and charge.

14. The method of Claim 4, wherein said first oligonucleotide is attached to a solid support.

15. The method of Claim 4, wherein said second oligonucleotide is attached to a solid support.

16. The method of Claim 2, wherein said cleavage agent comprises a structure-specific nuclease.

17. The method of Claim 16, wherein said structure-specific nuclease
5 comprises a thermostable structure-specific nuclease.

18. The method of Claim 2, wherein said cleavage agent comprises an enzyme, wherein said enzyme comprises a heterologous functional domain, wherein said heterologous functional domain provides altered functionality in a nucleic acid cleavage
10 assay.

19. The method of Claim 18, wherein said enzyme comprises a 5' nuclease.

20. The method of Claim 19, wherein said 5' nuclease comprises a
15 thermostable 5' nuclease.

21. The method of Claim 18, wherein said enzyme comprises a polymerase.

22. The method of Claim 21, wherein said polymerase is altered in sequence
20 relative to a naturally occurring sequence of a polymerase such that it exhibits reduced DNA synthetic activity from that of the naturally occurring polymerase.

23. The method of Claim 21, wherein said polymerase comprises a thermostable polymerase.
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24. The method of Claim 23, wherein said thermostable polymerase comprises a polymerase from a *Thermus* species.

25. The method of Claim 23, wherein said *Thermus* species is selected from
30 *Thermus aquaticus*, *Thermus flavus*, *Thermus thermophilus*, *Thermus filiformis*, and *Thermus scotoductus*.

26. The method of Claim 18, wherein said heterologous functional domain comprises an amino acid sequence that provides an improved nuclease activity in said nucleic acid cleavage assay.

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27. The method of Claim 18, wherein said heterologous functional domain comprises an amino acid sequence that provides an improved substrate binding activity in said nucleic acid cleavage assay.

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28. The method of Claim 18, wherein said heterologous functional domain comprises an amino acid sequence that provides improved background specificity in said nucleic acid cleavage assay.

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29. The method of Claim 18, wherein said heterologous functional domain comprises two or more amino acids from a polymerase domain of a polymerase.

30. The method of Claim 29, wherein at least one of said two or more amino acids is from a palm region of said polymerase domain.

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31. The method of Claim 29, wherein at least one of said two or more amino acids is from a thumb region of said polymerase domain.

32. The method of Claim 29, wherein said polymerase comprises *Thermus thermophilus* polymerase.

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33. The method of Claim 29, wherein said two or more amino acids from said polymerase domain comprise two or more amino acids from amino acids 300-650 of SEQ ID NO:1.

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34. The method of Claim 29, wherein said enzyme comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:2-68, 341, 346, 348, 351,

353, 359, 365, 367, 369, 374, 376, 380, 384, 388, 392, 396, 400, 402, 406, 408, 410, 412,
416, 418, 420, 424, 427, 429, 432, 436, 440, 444, 446, 448, 450, 456, 460, 464, 468, 472,
476, 482, 485, 488, 491, 494, 496, 498, 500, 502, 506, 510, 514, 518, 522, 526, 530, 534,
538, 542, 544, 550, 553, 560, 564, 566, 568, 572, 574, 576, 578, 580, 582, 584, 586, 588,
5 and 590.

35. The method of Claim 29, wherein said enzyme is encoded by a nucleic
acid selected from the group consisting of SEQ ID NOs:69-135, 340, 345, 347, 350, 352,
358, 364, 366, 368, 373, 375, 379, 383, 387, 391, 395, 399, 401, 405, 407, 409, 411, 415,
10 417, 419, 423, 426, 428, 431, 435, 439, 443, 445, 447, 449, 452, 454, 455, 459, 463, 467,
471, 475, 481, 484, 495, 497, 499, 501, 505, 509, 513, 517, 521, 525, 529, 533, 537, 541,
543, 549, 552, 559, 563, 565, 567, 571, 573, 575, 577, 579, 581, 583, 585, 587, and 589.

36. The method of Claim 8, further comprising the steps of forming a second
15 invasive cleavage structure comprising said non-target cleavage product and cleaving
said second invasive cleavage structure.

37. The method of Claim 36, wherein said invasive cleavage structure or said
second invasive cleavage structure comprises an oligonucleotide comprising a sequence
20 selected from the group consisting of SEQ ID NO:709-2640.

38. The method of Claim 36, wherein said invasive cleavage structure or said
second invasive cleavage structure comprises an oligonucleotide comprising a sequence
selected from the group consisting of SEQ ID NO:169-211 and 619-706.

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39. The method of Claim 1, wherein said RNA target nucleic acid comprises a
cytochrome P450 RNA.

40. The method of Claim 1, wherein said RNA target nucleic acid comprises a
30 cytokine RNA.

41. The method of Claim 3, wherein a portion of said target nucleic acid consisting of said first region and said second region of said target nucleic acid comprises a splice junction.

5 42. The method of Claim 3, wherein said first region or said second region of said target nucleic acid comprises an exon.

43. The method of Claim 3, wherein said first region or said second region of said target nucleic acid comprises an intron.

10 44. The method of Claim 1, wherein said RNA target nucleic acid is provided in a cell lysate.

45. The method of Claim 6, wherein said first oligonucleotide is covalently
15 attached to said second oligonucleotide.

46. A kit for performing an invasive cleavage assay comprising a probe oligonucleotide and an invasive oligonucleotide configured to form an invasive cleavage structure in the presence of an RNA target nucleic acid.

20 47. The kit of Claim 46, wherein said kit comprises an oligonucleotide comprising a sequence selected from the group consisting of SEQ ID NO:709-2640.

48. The kit of Claim 46, wherein said kit comprises an oligonucleotide
25 comprising a sequence selected from the group consisting of SEQ ID NO:169-211 and 619-706.

49. A method for detecting the presence of two or more target nucleic acid sequences comprising:

- 30 a) cleaving two or more invasive cleavage structures, each of said two or more invasive cleavage structures comprising an RNA target nucleic

- acid having a target sequence, wherein each of said invasive cleavage structures comprises a different RNA target sequence; and
- b) detecting the cleavage of said invasive cleavage structure.